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INFECTIOUS DISEASE

JC polyomavirus mutants escape antibody-mediated neutralization

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JC polyomavirus (JCV) persistently infects the urinary tract of most adults. Under conditions of immune impairment, JCV causes an opportunistic brain disease, progressive multifocal leukoencephalopathy (PML). JCV strains found in the cerebrospinal fluid of PML patients contain distinctive mutations in surface loops of the major capsid protein, VP1. We hypothesized that VP1 mutations might allow the virus to evade antibody-mediated neutralization. Consistent with this hypothesis, neutralization serology revealed that plasma samples from PML patients neutralized wild-type JCV strains but failed to neutralize patient-cognate PML-mutant JCV strains. This contrasted with serological results for healthy individuals, most of whom robustly cross-neutralized all tested JCV variants. Mice administered a JCV virus-like particle (VLP) vaccine initially showed neutralizing "blind spots" (akin to those observed in PML patients) that closed after booster immunization. A PML patient administered an experimental JCV VLP vaccine likewise showed markedly increased neutralizing titer against her cognate PML-mutant JCV. The results indicate that deficient humoral immunity is a common aspect of PML pathogenesis and that vaccination may overcome this humoral deficiency. Thus, vaccination with JCV VLPs might prevent the development of PML.

INTRODUCTION

JC polyomavirus (JCV) is a nonenveloped DNA virus that persistently infects the urinary tract of most adults. Although JCV infection is not known to be associated with overt clinical symptoms in healthy individuals, under conditions of immune dysfunction, such as HIV/AIDS, the virus can cause an opportunistic brain disease, progressive multifocal leukoencephalopathy (PML) [reviewed in (*1*, *2*)]. In recent years, PML has also been increasingly observed in patients treated with newer immunomodulatory drugs, such as the monoclonal antibody (mAb) therapeutics natalizumab and rituximab (*3*). The mechanisms through which a common, seemingly benign viral infection leads to lethal brain disease in a minority of immunodeficient individuals remain unclear.

A recently approved enzyme-linked immunosorbent assay (ELISA)–based test that detects serum antibodies specific for the JCV major capsid protein VP1 is used in clinical practice for PML risk stratification (*4*, *5*). About 1% of JCV ELISA-seropositive individuals develop PML during long-term natalizumab therapy (*6*, *7*). It is unclear why the JCV virion-specific antibodies detected in the ELISA fail to prevent or limit the development of PML. A possible explanation is that some or all of the antibodies detected in the ELISA fail to functionally neutralize the infectivity of the virus (*8*).

A series of reports have shown that JCV variants found in the cerebrospinal fluid (CSF) of PML patients carry a defined spectrum of mutations in portions of VP1 that form exposed loops on the surface of the assembled virion (*9*–*13*). Most PML-associated VP1 mutations disrupt the ability of the virion to bind sialylated glycans, which are thought to serve as infectious entry receptors for wild-type JCV genotypes typically found in the urine. Maginnis and colleagues have shown that PML-associated mutations disrupt the ability of JCV to infect five transformed cell lines (*14*). The findings led the authors to claim that PML-mutant JCV strains are globally noninfectious on all cell types. In conflict with this claim, Kondo and colleagues have recently shown that PML-mutant JCV strains readily infect primary human oligodendrocytes, astrocytes, and glial progenitor cells, both in culture and in intact brain tissue in vivo (*15*). In a commentary on the findings of Kondo and colleagues, Haley and Atwood speculate that primary glial cells support an alternative sialic acid–independent infection pathway that is presumably absent in some cell lines (*16*). Indeed, a variety of alternative entry factors have previously been proposed for various polyomaviruses [reviewed in (*17*)]. Using JCV reporter vectors (pseudoviruses), we identified several previously untested cell lines that are, like the primary glial cells studied by Kondo and colleagues, permissive for the infectious entry of both urine-derived wild-type and PML-mutant JCV genotypes (see the Supplementary Materials).

The availability of cell lines permissive for transduction with pseudoviruses representing PML mutants provided us with a tractable method for performing high-throughput serological analysis of JCV-neutralizing antibodies. Here, we use this system to perform functional neutralization serology to compare humoral immunity against JCV in healthy subjects and in patients suffering from PML.

Virus-like particle (VLP) vaccines can be remarkably effective for eliciting diverse, high-titer serum antibody responses capable of cross-neutralizing closely related viral serotypes (*18*–*20*). Neutralization serology was used to test the ability of an experimental VLP vaccine to elicit antibody responses capable of cross-neutralizing PML-mutant JCVs in a mouse model system and in a single case study of a PML patient administered an experimental JCV VLP vaccine.

RESULTS

Neutralization testing of sera from healthy human subjects

JCV neutralization assays were used to screen a panel of sera from 96 healthy adult subjects. Sixty (63%) of the subjects neutralized a

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2A (WT)	3B (WT)	3B-55F	3B-265S	2A-267F	2A-269F	GCN1
5.4	5.6	5.0	4.7	4.7	4.1	6.2
5.3	4.8	5.2	4.6	4.2	4.1	5.8
5.0	5.5	5.0	5.4	4.2	3.9	5.8
4.9	2.7	4.1	4.4	4.1	5.1	4.8
4.8	5.2	4.1	3.8	3.7	3.6	5.5
4.7	5.3	5.0	4.6	4.1	3.6	5.7
4.7	4.2	3.3	3.4	4.5	3.9	4.9
4.5	4.3	5.0	4.1	4.2	3.9	5.1
4.5	3.9	3.5	3.7	3.9	3.3	4.6
4.5	4.7	4.6	4.8	4.0	3.6	7.0
4.5	4.6	2.4	3.6	3.9	3.5	5.6
4.4	4.1	3.9	1.7	1.7	1.7	4.3
4.4	2.3	2.5	2.4	4.1	4.0	4.5
4.3	3.8	3.2	2.7	3.2	3.4	4.5
4.3	5.0	4.1	4.4	3.9	3.6	5.6
4.2	4.6	4.1	4.2	4.0	3.3	5.3
4.2	4.7	3.8	3.2	3.6	3.9	5.1
4.1	3.6	4.0	3.5	3.5	2.7	6.9
4.1	3.6	3.7	4.1	4.0	4.1	4.8
4.0	2.7	4.1	4.4	4.1	5.1	4.8
4.0	3.5	3.2	3.7	4.0	3.3	4.6
4.0	4.0	3.6	4.6	3.8	3.5	4.9
4.0	3.9	4.2	4.1	3.8	4.5	4.5
3.9	3.9	3.8	3.6	3.2	2.6	4.5
3.9	4.3	3.7	4.0	4.3	4.0	4.2
3.9	3.3	3.0	4.5	3.7	3.7	5.2
3.9	3.5	4.5	3.9	3.9	3.5	4.5
3.9	3.7	3.9	3.6	3.8	3.4	4.7
3.9	3.2	3.9	4.0	3.8	3.4	4.6
3.8	3.0	3.6	3.6	4.0	3.9	5.3
3.8	4.4	4.4	3.7	3.8	3.9	4.4
3.7	4.3	4.0	3.0	2.9	3.3	4.8
3.7	4.2	4.3	3.7	4.0	3.4	4.7
3.7	3.6	3.7	3.6	3.5	3.3	4.6
3.7	3.4	3.8	3.6	3.4	3.6	4.9
3.6	3.5	3.3	3.4	3.3	2.7	4.9
3.6	3.7	3.0	3.0	3.3	2.8	4.0
3.5	3.5	3.4	3.0	4.0	2.2	5.3
3.5	4.4	3.2	3.1	3.6	3.5	4.6
3.4	3.6	3.9	4.3	4.0	3.6	4.6
3.3	3.9	4.1	4.0	3.9	2.2	5.1
3.3	3.4	2.9	2.4	3.6	2.6	3.6
3.3	1.7	2.4	2.5	3.7	2.6	3.8
3.2	3.0	2.4	2.7	3.8	1.7	5.3
3.2	3.8	3.2	4.3	2.5	3.6	5.3
3.2	2.6	1.7	1.7	3.9	1.7	2.6
3.1	3.3	3.2	2.8	3.8	1.7	4.0
3.1	3.9	3.6	4.0	3.7	2.3	4.2
3.0	3.5	3.9	2.8	3.9	1.7	6.1
3.0	3.3	4.7	3.9	3.1	3.2	3.2
3.0	3.4	4.5	3.7	3.3	3.2	5.8
2.9	3.4	3.1	2.8	3.0	1.8	4.1
2.8	3.1	2.6	2.9	2.2	2.3	3.4
2.7	2.8	1.7	2.4	3.6	1.8	4.2
2.7	3.1	2.6	2.3	3.2	1.7	3.8
2.6	2.7	2.3	2.2	3.4	1.7	3.9
2.6	3.2	3.0	2.5	2.5	2.3	5.1
2.5	2.0	1.7	2.9	3.1	1.7	3.9
2.4	2.0	1.7	1.7	3.8	1.7	4.1
2.3	2.7	1.7	1.7	1.7	2.2	2.8

≤2.0 2.1–2.3 2.4–2.9 3.0–3.9 ≥4.0

Fig. 1. JCV neutralization serology (healthy adults). Serum samples from 96 individual adult subjects (rows) were serially diluted and initially tested for neutralization of a wild-type JCV-2A pseudovirus. Sixty serum samples that detectably neutralized JCV-2A were tested against additional JCV pseudoviruses. Sera that failed to detectably neutralize JCV-2A were excluded from further analysis and are not shown in the figure. The inverse $\log_{10}$ of the calculated EC_{50} is indicated with a color code. EC_{50} values ≤2 (blue cells) are considered neutralization-negative. For the precise VP1 sequence identity of JCV pseudovirus, see table S1.

pseudovirus based on a urine-derived wild-type genotype 2A JCV with a reciprocal 50% neutralizing titer (EC_{50}) of greater than 100 (Fig. 1 and table S1). This dilution was chosen as a seropositivity cutoff based on past evidence that serum dilutions of less than 1:100 can have nonspecific neutralizing effects (perhaps due to the effects of serum factors on the cultured cells) (*21*). With respect to the current study, it is important to note that, in healthy subjects, immunoglobulin G (IgG) antibody concentrations in the CSF are typically 200- to 500-fold lower than in the serum (*22*, *23*). Experiments with intravenously administered mAb therapeutic agents indicate that serum IgG antibodies chronically leak across the human blood-brain barrier and accumulate in the central nervous system (CNS) at low levels (*24*). In this model, IgG antibodies in the CSF essentially represent a lower-concentration snapshot of antibodies found in the periphery. Thus, individuals with a serum EC_{50} neutralizing titer of 100 would be expected to have poorly neutralizing or nonneutralizing concentrations of antibodies in their CNS. Our seroprevalence results using a neutralizing titer cutoff of 100 are concordant with a large body of previous seroprevalence studies using JCV-1A VP1 ELISA methods [reviewed in (*2*)].

Although most of the samples that neutralized the 2A pseudovirus also neutralized all other tested JCV genotypes with similar titers, a minority of sera failed to detectably neutralize one or more PML-mutant pseudoviruses (Fig. 1). Eleven of the 60 serum samples that neutralized the 2A pseudovirus failed to neutralize the 2A-269F pseudovirus. The S269F mutation represents the most common variant observed in the CSF of PML patients (*11*, *12*). The results are consistent with the idea that VP1 mutations found in the CSF of PML patients could confer a selective advantage to the virus by allowing escape from the apparently restricted spectrum of JCV-neutralizing antibodies observed in a minority of JCV-seropositive individuals.

Longitudinal neutralization analysis of PML patient plasma

To investigate the idea that the unusual phenotype of having JCV neutralization "blind spots" might be associated with an increased risk of developing PML under conditions of T cell immunodeficiency, we performed neutralization serology on a panel of plasma samples from PML patients. This work was confronted by two theoretical issues. First, PML patients might exhibit a narrow neutralization blind spot encompassing only the specific JCV VP1 sequence found in their CSF during PML. Second, it seems conceivable that neutralization blind spots might close during or after the development of PML. These considerations restricted our focus to patients for whom plasma samples had been collected and archived before the diagnosis of PML, and for whom the JCV sequences found in their CSF during PML were known. Six PML patients met these criteria. The underlying immunodeficiency in each of the six patients was HIV/AIDS, which was treated with combination antiretroviral therapy (table S2).

Pseudoviruses were constructed to represent the cognate mutant VP1 sequence found in individual patients' CSF during PML (table S1). Pseudoviruses representing inferred wild-type VP1 sequences were also produced. In some instances, the patient plasma samples were tested against near-cognate pseudoviruses. As shown in Fig. 2, fig. S4, and table S3, all six PML patients exhibited little or no neutralization of their cognate PML-mutant pseudovirus at time points before PML diagnosis, even when there was robust neutralization of the wild-type pseudovirus. The results confirm the hypothesis that PML-specific VP1 mutations can allow the virus to escape from antibody-mediated neutralization.

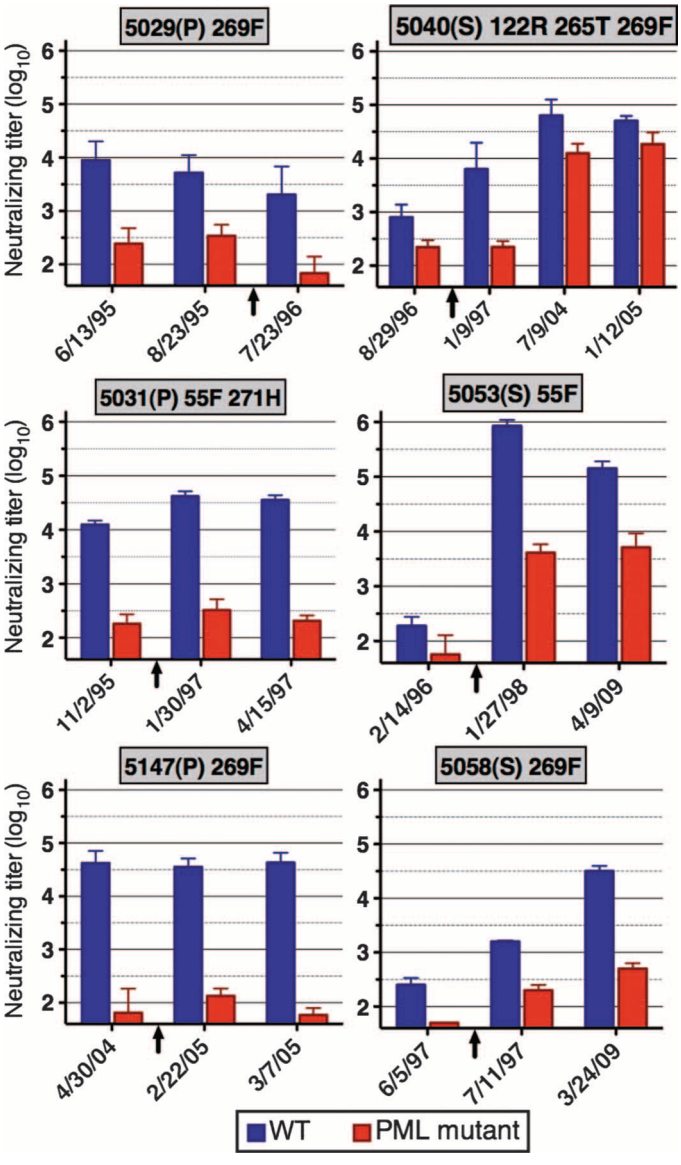

Fig. 2. PML patient neutralization serology. Pre- and post-PML plasma samples from six patients were tested for neutralization of cognate (or near-cognate) wild-type (WT) and PML-mutant pseudoviruses. Specifically, patient 5029 was tested against viruses 5029w (cognate WT, blue) and 5029m (cognate mutant, red); 5031: 5031w/5031mb; 5040: 2A/5040m; 5053: 5053w/5053m; 5058: 2A/5147m; 5147: 5147w/5147m (see table S1). Testing of PML patient sera against additional pseudoviruses is shown in fig. S4. Patients whose disease progressed (left column) are indicated with (P), and patients who survived (right column) are indicated with (S). Dominant PML-associated mutations observed in each patient's CSF are indicated. *Y* axes indicate neutralizing EC_{50}. Error bars represent SEM for data from three independent experimental replicates, two of which were performed with blinding. Arrows below the *x* axes indicate the date of onset of PML symptoms. Date format is month/day/year.

Patients who survived PML eventually developed broader antibody responses capable of neutralizing their cognate PML-mutant pseudovirus. This suggests that at least some individuals with neutralization blind spots are ultimately capable of mounting broadly cross-neutralizing antibody responses. In contrast to patients who survived PML, the three patients with progressive (fatal) disease did not develop the ability to robustly neutralize their cognate mutant virus. Although this could simply reflect the recovery of effective cell-mediated immunity (which would, in turn, provide $CD4^+$ T cell help for B cell responses), an important conclusion that can be drawn from the result is that individuals with JCV-neutralizing blind spots are not intrinsically incapable of mounting more broadly neutralizing antibody responses.

Remarkably, plasma from patients 5029 and 5058 robustly neutralized the 2A-269F pseudovirus at time points where there was poor neutralization of the patient-cognate mutant virus, which carries the S269F mutation in a slightly different genotypic background (fig. S4 and table S2). The results suggest that naturally occurring wild-type genotypic variations outside the PML mutation "hotspots" can influence neutralization-escape phenotypes. This is consistent with the observation that a few healthy subject sera that robustly neutralized the wild-type 2A pseudovirus showed very low titers against the wild-type 3B pseudovirus (Fig. 1). Together, the results illustrate the caveat that it is essential to analyze the neutralization of the exact VP1 sequence(s) observed in any given subject.

Evaluation of JCV VLPs as vaccine immunogens in a mouse model

To test the idea that a VP1 VLP-based vaccine against JCV might elicit broadly neutralizing serum antibody responses, groups of mice were given a single intramuscular dose of 720 ng of a monovalent VLP preparation in alum. A single priming dose of VLP immunogen elicited high-titer serum antibody responses capable of robustly neutralizing the cognate pseudovirus (Fig. 3). Each set of primed mice failed to robustly cross-neutralize at least one noncognate pseudovirus type. This result recapitulates the neutralization blind spot effects observed in human subjects.

Mice were administered a booster dose of the same monovalent VLP preparation. Sera from all boosted animals cross-neutralized all tested JCV variants (fig. S5). This shows that blind spots can be closed through prime-boost vaccination with a monovalent JCV VLP vaccine. Overall, the wild-type 2A VLPs elicited the most uniformly robust cross-neutralizing responses, suggesting that it is unnecessary (and perhaps undesirable) to use PML-mutant VLP immunogens to elicit antibodies capable of neutralizing PML-mutant pseudoviruses (*25*).

Case study of a PML patient administered an experimental JCV vaccine

PML patient 5228 is a 75-year-old female with idiopathic CD4 lymphopenia who was admitted at the Department of Infectious Diseases of San Raffaele Hospital (Milan, Italy) upon diagnosis with PML on 24 May 2012. Her case has not previously been reported. The patient's clinical condition deteriorated rapidly after admission and she became comatose. In addition to mefloquine and mirtazapine, the patient was treated with a previously reported vaccination protocol consisting of separate administrations of interleukin-7 (IL-7) and JCV-1A VLPs combined with imiquimod (*26*). As shown in Fig. 4 and table S3, vaccination was followed by a roughly 100-fold increase in the patient's neutralizing titer against her cognate mutant virus. This confirms that PML patients are not intrinsically incapable of mounting new antibody responses capable of neutralizing their cognate JCV strain. After vaccination, patient 5228 showed an extraordinarily high peak titer (25 million) against her inferred wild-type JCV. This is particularly remarkable in the sense that the patient

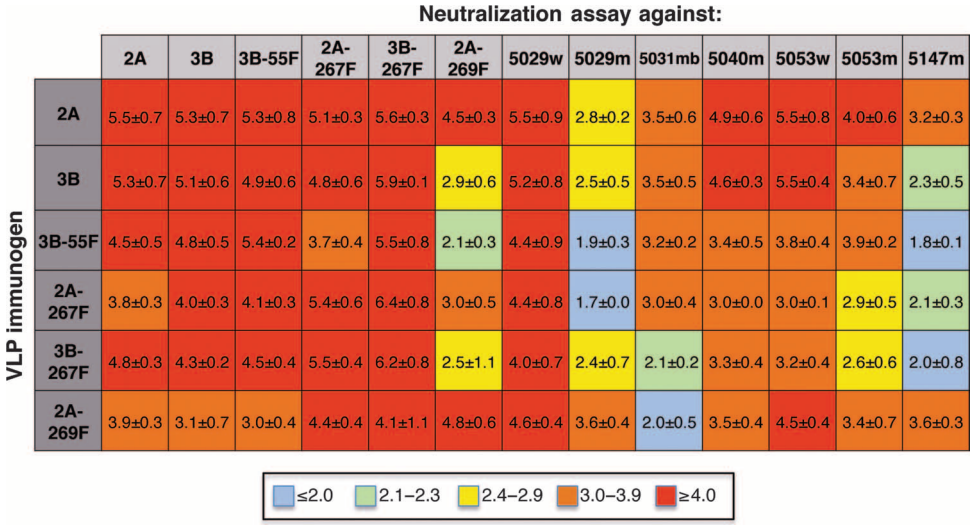

Fig. 3. Preclinical evaluation of a candidate JCV VLP vaccine. Mice were given an intramuscular injection of VLPs based on the JCV genotype indicated in each row label. Four weeks later, sera from the mice were tested for neutralization of various pseudoviruses indicated in column labels. Numerical values represent EC_{50} neutralizing titers. Error represents SD for independent neutralization assays of sera from five replicate mice. Preimmune sera were nonneutralizing at a 1:100 dilution.

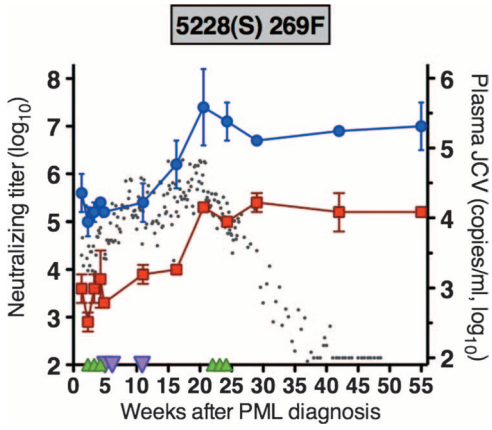

Fig. 4. JCV-neutralizing titers after vaccination of PML patient 5228. Patient 5228 was administered JCV VLPs subcutaneously at three time points (downward purple triangles). Recombinant IL-7 was also administered for two cycles each consisting of a subcutaneous weekly dose of 10 μg/kg (upward green triangles). The patient's neutralizing titer against her cognate PML-mutant JCV (red squares) or inferred WT JCV (blue circles) was monitored over time. JCV load in the patient's plasma (gray dots) was also monitored over time. See the Supplementary Materials for additional patient details.

was suffering from intermittent lymphopenia at the time of vaccination (fig. S6). Although it is tempting to speculate that the JCV VLP component of the treatment was a primary factor in induction of a potent humoral immune response, we note that a previous case study of a PML patient administered recombinant IL-7 alone (without JCV VLPs) also showed increasing anti-JCV antibody titers, including a pronounced IgM response (*27*). This suggests that improved CD4 T cell function and "auto-inoculation" with PML lesion–derived virions could have played a significant role in the response. Increases in patient 5228's JCV-neutralizing titer preceded a gradual fall in JCV viremia (Fig. 4 and table S5) and an arrest of PML lesion progression (Fig. 5). It is uncertain whether there was a causal relationship between the improved JCV-neutralizing antibody response and the resolution of disease progression.

DISCUSSION

Our results indicate that a minority of healthy JCV-seropositive subjects are deficient in serum antibodies capable of neutralizing JCVs carrying VP1 mutations associated with PML. A simple explanation for this finding is that some individuals may have an unusually low diversity of plasma cells secreting effectively neutralizing antibodies, such that single point mutations in VP1 can allow the virus to evade neutralization. This finding is consistent with past observations in murine viral challenge systems (*28*), and we have recently reported a similar scenario for BKV (a close relative of JCV) (*29*). Neutralization-escape mutations would presumably increase the fitness of the virus under circumstances where T cell–mediated immunity in the CNS is impaired, and antibody-mediated neutralization therefore serves as a last line of defense against neuropathic JCV replication. The idea that humoral vulnerability is a key element of PML pathogenesis is supported by the observation that all six PML patients we studied displayed neutralization blind spots before disease onset.

At present, there are no antiviral agents known to be effective for the treatment of JCV disease, and reversal of immune dysfunction remains the only approach of proven utility for the treatment of PML. Immune reconstitution treatments are not always timely or successful and, even if they are successful, sometimes lead to immune reconstitution inflammatory syndrome, which can be lethal (*30*). VLP-based vaccines against other viral families have been highly successful in humans (*31*, *32*). In particular, VLP-based vaccines against human papillomaviruses (which share key structural features with polyomaviruses) elicit remarkably potent, diverse, and long-lasting neutralizing antibody responses [reviewed in (*20*)]. Our testing of experimental JCV VLP immunogens demonstrates that high-titer serum antibody responses capable of broadly cross-neutralizing PML-mutant JCVs can be elicited by prime-boost vaccination with VLPs representing a single wild-type JCV genotype. Because antibodies are present at much lower levels in the CNS (*23*, *24*), the apparently high immunogenic potency of JCV VLP immunogens could be important for induction of protective levels of JCV-neutralizing antibodies in the brain parenchyma. Together, the results suggest that a prophylactic JCV VLP vaccine could serve to boost and broaden humoral immunity against JCV and thus protect at-risk individuals against the development of PML.

Although the widespread availability of combination antiretroviral therapy has led to a marked decrease in the incidence of AIDS, treatment failure and/or lack of compliance with antiretroviral dosing schedules remain long-term risks for HIV-infected individuals. The availability of a preventive vaccine against PML could thus be of potential benefit for people living with HIV.

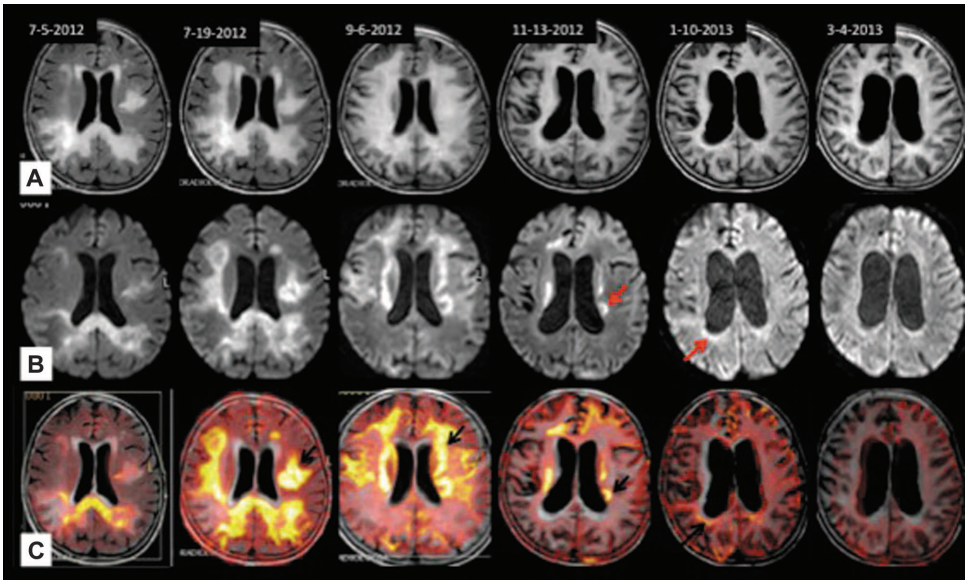

Fig. 5. Evolution of PML lesions by magnetic resonance imaging in patient 5228. The patient was initially diagnosed with PML on 24 May 2012. The date of each scan is indicated along the top of the figure. Axial fluid-attenuated inversion recovery (FLAIR, row a), diffusion-weighted imaging (DWI, row b), and FLAIR-DWI merged images (row c) show the evolution of PML lesions. Row a: The first image on the left (July 2012) shows a high signal intensity lesion of the right parieto-occipital white matter extending contralaterally through the corpus callosum; another focus is present in the left subcortical temporal region. Subsequent examinations showed evolution of the signal alteration with progressive rapid extension of the lesions to the entire white matter bilaterally. In September 2012, the white matter was completely occupied by lesional and atrophic processes and enlargements of the ventricles and cortical sulci began to appear. The last examinations (January and March 2013) show progression of atrophy. Row b: Axial DWI images show the evolution of the hyperintense signal alterations corresponding to the front of progression of PML lesions (red arrows). The signal alteration is substantially reduced starting from November 2012 and is no longer visible at the March 2013 examination, implying lesion stabilization. Row c: Merged FLAIR and DWI sequences show, in yellow (black arrows), the initial fronts of advance of PML lesions and their regression over time.

At present, treatment of multiple sclerosis patients with the highly effective mAb therapeutic natalizumab is generally time-limited because of the progressively increasing risk of PML side effects (*2*). The utility of the drug rituximab for treating rheumatoid arthritis is likewise limited by PML side effects. Efalizumab, an immunomodulatory mAb previously used for the treatment of psoriasis, was withdrawn from market because of PML side effects. Perhaps a dozen other immunomodulatory agents that are either in development or on the market are potentially associated with rare PML side effects. Thus, the availability of safe and effective measures for preventing PML could significantly increase the overall safety profiles of therapies for a wide variety of autoimmune diseases and lymphoid cancers. This would be particularly true for immunomodulatory therapies, such as natalizumab, that are compatible with vaccination (*33*).

A limitation of our study is the availability of only a small population of PML patients with appropriate longitudinal samples. Thus, it remains unclear whether neutralizing blind spots are a universal feature of PML development or whether, in some patients, the disease develops despite the presence of an effective humoral response. Likewise, although the single case study of patient 5228 is consistent with the possibility that IL-7/JCV VLP administration was a factor in arrest of PML progression, the fact that other patients, including another patient administered IL-7 without VLPs (*27*, *34*), sometimes spontaneously recover leaves the question of whether JCV VLPs would be a useful therapeutic intervention for the treatment of PML unclear.

An additional limitation of the study is that we did not have access to paired CSF samples for healthy subjects and only sporadic CSF samples from some of the PML patients. In the future, it will be important to test the prediction that serum JCV-neutralizing antibody responses essentially represent a 200- to 500-fold higher titer than would typically be observed in CSF (*22*–*24*). It will be important to address this question using passive transfer of antibodies (or bone marrow plasma cells) in the new murine PML model reported by Kondo and colleagues (*15*). Such experiments could test the prediction that broadly cross-neutralizing antibodies could protect mice against PML when administered at high doses, whereas lower doses of "blind spot" polyclonal antibodies (for example, from mice given a single priming dose of JCV-3B VLPs) might drive the evolution of the common L55F and S269F escape mutations.

MATERIALS AND METHODS

Study design

A set of 96 previously characterized (*21*) healthy human sera were screened for neutralization of wild-type JCV-2A pseudovirus. The JCV-2A–neutralizing sera were then tested against diverse range of JCV variants. Testing of healthy subject sera did not use blinding. Serum samples from six PML patients for whom CSF JCV sequences were known were initially tested against the indicated cognate pseudoviruses without blinding. Samples from the six PML patients were then randomized and retested in two independent additional experiments in a blinded fashion. Neutralization serology for patient 5228 (who was vaccinated with JCV VLPs) was likewise initially performed without blinding followed by two blinded repeats. Experiments with mice arbitrarily used five animals per group. Serological analysis of mice was performed without blinding.

Pseudovirus production

Pseudovirions were produced using previously described methods, with minor modifications (*21*, *35*). Briefly, *Gaussia* luciferase reporter genes were packaged into pseudovirions in 293TT cells transiently transfected with JCV VP1/2/3 expression plasmids. The resulting pseudovirions were purified over OptiPrep gradients. Additional methodological details are presented in the Supplementary Materials.

Sera

Healthy subject sera were purchased from Equitech Bio Inc. and Innovative Research Inc. (*21*, *29*, *36*). Ethical assurances are provided on the

suppliers' Web sites. Samples from PML patients were collected either for diagnostic purposes or as part of internal research protocols approved by the Ethical Committee of San Raffaele Hospital, Milan. Written informed consent to use of samples and clinical information was given by patients who were still alive at the time of the start of the study. Permission from the Ethical Committee was given for the use of samples and clinical information from patients who were deceased.

Serum and plasma samples were heat-inactivated at 56°C for 30 min, followed by brief centrifugation to sediment any aggregated material. For samples from healthy adults, serum IgG antibodies were then purified out of the serum samples using Melon Gel (Pierce) resin according to the manufacturer's instructions. Plasma samples from PML patients and mice were not subjected to Melon Gel purification.

Neutralization assays

Generation and use of ART cells [an ovarian cancer line (*37*, *38*)] and SFT cells [a gliosarcoma line (*39*)] for neutralization assays have been described previously (*29*). In initial validation experiments, comparable JCV neutralization results were observed using either cell line (fig. S3) (*39*). Neutralization serology studies used ART cells with the dose of VP1 specified in table S1.

Preclinical VLP vaccine

Animal experiments were performed at the National Cancer Institute facilities under the approval of the Animal Care and Use Committee and according to the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International. Procedures were carried out in accordance with the eighth edition of the National Research Council of the National Academies' *Guide for the Care and Use of Laboratory Animals*. Female BALB/cAnNCr mice were subjected to intramuscular immunization with 720 ng of JCV VLPs (VP1 only) mixed with 0.2% of aluminum hydroxide (alum, InvivoGen) with a total volume of 50 μl. Five mice were immunized per JCV VLP type. One month after the single priming dose of VLPs, plasma samples were collected by submandibular bleed into Microtainer lithium-heparin tubes (BD). The mice were then boosted with the same JCV VLP type intramuscularly in alum. Serum samples were collected 1 month after the booster dose.

Vaccination case study

Ethical approval for compassionate administration of the IL-7/VLP combination treatment was provided by the Ethical Committee of San Raffaele Hospital. The family of patient 5228 gave written informed consent for the collection and use of research samples and for the experimental administration of the IL-7/JCV VLP vaccine. Recombinant human IL-7 was given subcutaneously at 10 μg/kg on 9, 16, and 23 June 2012 (first cycle) and on 25 October, 2 November, and 9 November 2012 (second cycle). Subcutaneous injections of 1 mg of VLPs composed of JCV-1A VP1 were performed on 28 June, 6 July, and 8 August 2012. The VLP preparation was the same as the one previously administered to two other patients whose cases were described in a recent report by Sospedra and colleagues (*26*). Imiquimod cream (5%, Aldara, Meda Pharmaceuticals) was applied as a vaccine adjuvant topically at the injection site. The treatments appeared to be well tolerated. Additional clinical details for patient 5228 are provided in the Supplementary Materials.

Statistical analyses

The neutralization titers were calculated in Prism (GraphPad) using nonlinear regression analyses. A sigmoidal dose-response equation (variable slope) (four-parameter logistic equation) was used with top and bottom values constrained based on "no antibody" and "no virus" controls, respectively.

SUPPLEMENTARY MATERIALS

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Text
Fig. S1. Transducibility of various cell lines.
Fig. S2. An example of luminometry results for a pilot JCV neutralization assay using ART cells.
Fig. S3. Neutralization assay validation.
Fig. S4. PML patient neutralization serology (an expansion of Fig. 2).
Fig. S5. Serological analysis of mice after a booster dose of JCV VLPs.
Fig. S6. JCV-neutralizing titers after vaccination of PML patient 5228 (an alternative version of Fig. 4).
Table S1. Characteristics of JCV pseudovirus stocks.
Table S2. Patient characteristics.
Table S3. PML patient neutralization serology (source data).
Table S4. Neutralization serology of patient 5228 (source data).
Table S5. Viremia of patient 5228 (source data).
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JC polyomavirus mutants escape antibody-mediated neutralization

Upasana Ray, Paola Cinque, Simonetta Gerevini, Valeria Longo, Adriano Lazzarin, Sven Schippling, Roland Martin, Christopher B. Buck and Diana V. Pastrana (September 23, 2015)
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Editor's Summary

Opportunity knocks for JC polyomavirus therapy

JC polyomavirus (JCV) can be found in the urinary tract in most adults, resulting in a persistent but asymptomatic infection. However, in immunocompromised individuals, JCV opportunistically infects the brain, resulting in the debilitating and frequently fatal disease progressive multifocal leukoencephalopathy (PML). No treatments are currently available for PML, but two papers now identify and exploit a gap in the immune response to JCV. Ray *et al.* report that JCV strains found in the cerebrospinal fluid of PML patients have mutations that prevent antibody neutralization and that these blind spots can be overcome with vaccination. Jelcic *et al.* suggest that broadly neutralizing antibodies derived from a patient who recovered from PML may fill this gap.

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